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INFLUENCEOF CRYOLIPOLYSIS ON LEPTIN REGULATION IN CENTRAL OBESE SUBJECTS

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ABSTRACT

Background: Obesity is a chronically disorders with excess body fat, which impairs the health of the person and is linked to comorbidities, such as diabetes mellitus, high blood pressure, and vascular disorder.**Aim**: To investigate the effect of cryolipolysis on leptin hormone in obese subjects. **Methods:** Thirty obese individuals were recruited from both sexes aged 30 to 40 years with a bodyweight index of 30 to 35. The control group pretest-posttest was employed. Experimental Group (A): composed of 15 obesity participants, received cryolipolysis, duration 60 min. 1 session/month for 3 months and diet intervention. Control Group (B): 15 obese individuals had a 3-month dietary procedure. All subjects have been examined with body mass index, hungerlevel, and serum leptin hormone before and after the intervention. **Results:**body mass index, hunger level, and leptin hormone in group (A) were significantly reduced more than group (B). The level of alpha ($P < 0.05$) has beenestablished.**Conclusion:** Theimplementation ofcryolipolysis technology on obese individuals has a beneficial effect on body mass index, hunger level, and leptin hormone.

Keywords: Cryolipolysis, Serum leptin hormone, Body Mass Index, Hunger level.

INTRODUCTION

Obesity can be defined as an excess of body fat **(1),**The effect of obesity is inequality of dietary input and energy consumption. **(2)**Obesity is an increasing worldwide health issue, and morbid obesity is rapidly increased.**(3),** The WHO classification using BMI defines obesity as ≥30 kg/m2, and ≥40 kg/m2 is considered extreme obesity **(4).**

The extended and near-term appetite control is implicated in two types of hormones; toning circulatory hormones like the insulin, leptin indicators for extended term energy state and are implicated in appetite prohibition, episodic or intestinal hormones," including cholecystokinin (CCK), GLP-1, and PYY ", are embroiled in appetite prohibition, only one episodic hormone, Ghrelin, is mired in appetite arousal.**(5)**

Fat removal and body remodeling are becoming ever more popular. Liposuction nowadays is the most frequent and effective body sculpting operation. Because of the intrusive nature and immanent hazards of liposuction, ongoing investigation into noninvasive procedures was conducted. Variable demonstrations of scientific efficiency have been conducted in several noninvasive technologies, including " laser utilization, ultrasound, radiofrequency and infrared light."**(6).**For the selective elimination of fatty cells, a noninvasive process was developed, which reduces the fat layers called "cryolipolysis." This nonsurgical method uses regulated cooling to reduce subdermal fat without harming the adjacent tissue. **(7).**

Design

Pre/ posttest randomized controlled trial design. Procedures followed agreed with "Institutional Ethical Committee Clearance, and written informed consent was taken from every case." Pan African Clinical Trial Registry number is (**PACTR202010677385435**).

Subjects:

Thirty central obese subjects were involved in this study; their age ranged from 30- 40 years; Subjects were divided into 2 groups experimental and control group

Group A: experimental group, 15 central obese subjects, their age ranged from 30- 40 years, they received cryolipolysis sessions with diet restriction by using low carbohydrate diet.

Group B: control group, 15 central obese subjects, their age ranged from 30- 40 years; they received diet restriction by using low carbohydrate diet.

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Inclusive criteria of Patient
30 central obese subjects, their ageswere from 30-40 years, their BMI was from 30-35kg/m2.

Exclusive criteria of Patient:

Smokers, pregnant women, Cardiovascular disease, Musculoskeletal disease, Physical disability, Renal and metabolic diseases, Patients receiving any drug therapy such as lipid lowering therapy, vitamins and antioxidants.

Intervention
Cryolipolysis:
In all cases, the cryolipolysis applicationuses a vacuum-based huge applicator (Cool Max) for the abdomen and vacuum medium applicators (either CoolCore or Cool- Curveþ) for flanks. (60 minutes session once a month for three successive months). **(8)**
Diet interventions: Trained professionals encouraged individuals at the low-carb diet to limit the intake of carbs to fewer than 20 g per day by a popular diet book issued by the lay press and supplementary materials**(9).**

Methods
Evaluation procedure:
"Weight, height, BMI, fat mass, waist-hip ratio, waist circumference, blood pressure, and radial pulse" will be evaluated before the beginning of the study by 48h by the main constructor. Weight, BMI, waist-hip ratio, and fat mass wereassessed by the inbody system. The circumference of the waist was evaluated by many anatomical sites, such as the halfway, between the lower rib and the crest of an iliac, the umbilicus, the narrowest or largest waist circumference, right under rib, and right over the crest of the iliac, "The NHLBI practical guide recommends measuring just above the iliac crest, Waist circumference dimension becomes achieved with the subject standing, nude midriff, after the subject exhales, with each foot touching and palms putting freely. The measuring strip becomes manufactured from a fabric that isn't easily stretched; it becomes positioned perpendicular to the long axis of the body and horizontal to the ground and implemented with tension; however, it becomes no longer positioned stress at the belly wall **(10).**
RESULTS
Statistic analyzed conduct using SPSS, "version 25(SPSS, Inc., Chicago, IL)". Alpha level set at 0.05, used for data analysis. Data obtained from the two groups regarding leptin, body mass index, and hunger level were calculated before starting cryolipolysis sessions and diet intervention later after finishing the experimental program for the two groups.as shown in table1

Table 1. Baseline demographic and clinical characteristics of subjects(N=30) *

Characteristics	"Study group " (n=15)	"Control Group " (n=15)
Age(years)*	33.48±3.91	33.75±4.21
Sex(M/F)	6/9	8/7
Weight(kg)*	86.15±5.6	84.6±4.98
Height(cm)*	163.3±5.92	163.7±4.74
Leptin (ng/ml) **	95.4(89,98)	91.4(88.2,96.2)
BMI(Kg/m^2) *	33.2±1.5	33.1±1.4
Hunger Level***	4	3

"BMI. Body mass Index * Data are mean± SD, **Median (inter quartile range)", ***Median

Differences between experimental and control groups: -

"A Mann-Whitney U test was run to determine if there were differences in" leptin, BMI and Hunger Level pre and post scores between experimental and control groups.
Leptin pre scores for experimental (mean rank =16.93) and control (mean rank =14.07) were not statistically significantly different, U =91, z = -0.98, p =0.39. While Leptin post scores for experimental (mean rank =10.13) and control (mean rank =20.87) were statistically significantly different, U =32, z = -2.34, p =0.0005. as shown in table 2
BMI scores for experimental (mean rank =16.13) and control (mean rank =14.87) were not statistically significantly different, U =103, z = -0.39, p =0.71. While BMI post scores for experimental (mean rank =9.27) and control (mean rank =21.73) were statistically significantly different, U =19, z = -3.88, p =0.0003. as shown in table 2

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Hunger scores for experimental (mean rank =16.5) and control (mean rank =14.5) were not statistically significantly different, U =97.5, z = -0.9, p =0.54. While Hunger post scores for experimental (mean rank =8.4) and control (mean rank =22.6) were statistically significantly different, U =6, z = -4.6, p =0.0001. as shown in table 2

Table2. Clinical Characteristics of Subjects after 3 months of intervention (N=30) *

Characteristics	"Study group" (n=15)	"Control Group" (n=15)	Mann-Whitney U	Z-Value	P Value
Leptin(ng/ml) **	45.1(23.5,76.1)	83.6(78,89)	32	-3.34	0.0005
BMI(Kg/m^2) *	29.3±1.3	32.2±2.5	19	-3.88	0.0001
Hunger Level***	1	3	6	-4.6	0.0001

BMI. Body mass Index * Data are mean± SD, **Median (inter quartile range), ***Median, P-Value < 0.05 indicate statistical significance

Table3. Clinical Characteristics of Subjects in the study group

Characteristics	Baseline	after 3 months	Mean Rank	Z-Value	P Value
Leptin(ng/ml)**	95.4(89,98)	45.1(23.5,76.1)	8	-3.41	0.001
BMI(Kg/m^2)*	33.2±1.5	29.3±1.3	8	-3.41	0.001
Hunger Level***	4	1	8	-3.52	0.0004

BMI. Body mass Index * Data are mean± SD, **Median (inter quartile range), ***Median, .P-Value < 0.05 indicate statistical significance

Table 4. Clinical Characteristics of Subjects in the control group

Characteristics	Baseline	after 3 months	Mean Rank	Z-Value	P Value
Leptin(ng/ml)**	91.4(88.2,96.2)	83.6(78,89)	8	-3.41	0.001
BMI(Kg/m^2)*	33.1±1.4	32.2±2.5	7.5	-2.56	0.01
Hunger Level***	3	3	4.5	-2.83	0.005

BMI. Body mass Index * Data are mean± SD, **Median (inter quartile range), ***Median. P-Value < 0.05 indicate statistical significance

DISCUSSION

The main aim of this study was to scrutinize the effect of cryolipolysis on leptin hormone in central obese subjects by measuring the body mass index and serum leptin.

This study was conducted on thirty obese subjects who were selected from the outpatient clinic of the Faculty of Physical Therapy, Cairo University, and agreed to participate in the study; their ages ranged from 30 to 40 years old and were suffered from obesity as their BMI from 30 to 35 kg/m^2.

Subjectswere randomlyassignedintotwogroups; each group consisted of 15 subjects.Experimental group(A), their mean age (40.6±3.05) years received cryolipolysis and diet intervention (60 minutes session once a month for three successive months).Control group(B), their mean of age (40.35±3.06) years, consisted of 15 subjects received diet intervention (for three consecutive months).

The subjects in both groups were assessed for BMI calculation and serum leptin level pre and post-treatment.

Within the limitations of this study, the results showed that post-treatment, there was a significant decrease in BMI in both groups. However, the reduction in BMI in-group (A) was statistically more than group (B). There was a significant decrease in serum leptin in both groups, but there was a significant decrease in serum leptin level in group (A) more than in group (B).

From statistical analysis of BMI and serum leptin, pre-and post-values in the experimental group (A) and control group (B).

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There was a decrease in BMI at the end of treatment in relation to pretreatment values, and this difference was significant. The improvement in serum leptin hormone was statistically significant in the experimental group (A) compared to the control group (B).

As far as we know, this is the first study evaluating the effect of cryolipolysis on leptin hormone in central obese subjects; there were few studies that investigated the influence of cryolipolysis on serum testosterone.

The current study results coincide with the findings of **Patricia F et al.2016**whoshowed a substantial decrease of the fat layer after 16 weeks of treatment via measuring circumference and ultrasonography. The volunteering patients in the current study have not changed their food and maintaining consistent body weight during therapy. In their investigation, **Ferraro et al.2012** reported that the bodyweight remained constant for all patients and powerfully suggested that local treatment led to a decline in fat ply**. (11)**

The present study's findings contradict the conclusions of **Catherine Hanna, 2019** who discovered that the paradoxical adipose hyperplasia is an infrequent but harmful side effect of cool sculpting. It mostly happens in guys. This results in fat cells growing larger instead of smaller at the treatment site. Why this happens is not entirely known. Although it is perhaps a cosmetic than a physiologically threatening side effect, adipose hyperplasia paradoxically does not disappear alone. **(12)**

The findings of **Ferraro et al.,2012** are in line with our investigation.that cryolipolysis has been claimed as a potent, noninvasive treatment of body reshaping. The authors featured that the abdomen was significantly reduced. **(13)**

Sheki et al.,2012identified the impact of cryolipolysis in a Chinese sample population for body reshaping. After two months of cryolipolysis therapy in the abdominal area, the authors documented a decline of 4.9 mm in fat thickness. (**14)**

Macedo et al.2012revealed that cryolipolysis is helpful in treating flanks or abdominal areas over-collected fatty tissues. **(15)**

Our study comes in agreement with**Mohamed Serag and Mohamed Elshafey 2016**who stated that cryolipolysis has a beneficial impact than Laser lipolysis in the reducing of "waist-hip ratio, skin folds at Suprailiac level and subcutaneous adipose tissue (SAT)"in BMI and bodyweight reduction, no substantial difference exists between them. All subjects have no impact on VAT (visceral adipose tissue). **(16)**

Our findings were in accordance with **Lilit Garibyan.2014** in relation to the outcomes of the group of Cryolipolysis. A considerable volume reduction occurred at 2 months following one single therapy of cryolipolysis to the flank.

. The average loss determined in absolute fat was 56.2 cc from handled flanks, and the untreated flanks were 16.6, which became highly significant ($P<0.001$) and represented a mean utter difference of 39.6 cc between the sides being treated and the sides being untreated. This data shows that, on average, fat volume reduction is about 40 % after 60 days following treatment after one cryolipolysis session. It seems that cryolipolysis is harmless and well tolerable. No discomfort, blisters, scar tissue, redness, or oedema have been recorded in participants. By 1 week after therapy, all individuals were free of pain. At the treatment site, all individuals observed a decline of feeling ten minutes after intervention. **(17)**

Finally, cryolipolysis is a well-tolerant, effective, and safe procedure for noninvasive fats elimination. These results are consistent with the findings of recent research by **Nils Krueger et al.2014**, who said that accurate use of cold temperatures causes the deaths of adipocytes afterward absorbed and destroyed by macrophages.Instantly following therapy, no alterations in subcutaneous fat are visible. An inflammatory stage driven by adipocyte apoptosis as represented by inflammatory cell inflow can be noticed 3 days after therapy and maximum about 2 weeks after therapy when “adipocytes are encircled with histiocytes, neutrophils, lymphocytes, and other mononuclear cells.”At 2 weeks to one month following the treatment, the lipid cells encircle, wrap and disintegrate by macrophages and other phagocytes as a natural body's reaction to harm. The inflammation reduces, and the adipocyte volume decreases four weeks following therapy. The interlobular septa are significantly thickened two to three months after treatment, and the inflammatory phase diminishes further. At that point, the volume of fat in the treated area appears to have fallen, and septae are the largest part of the volume of tissues. **(18)**

Moreover, it agreed with **Stevens WG et al. 2013** which notified that cryolipolysis is harmless and suitable for repeat applications for all skin types without any observed pigmentation abnormalities. Those doing regular physical activity, consume a nutritious diet, have evident fat folds in the trunk, are genuine in expectation, and being willing to preserve the effects of Cryolipolysis in a healthful, active way of life are the best nominee. **(19)**

In this paper, **Saski GH et al.2015** have validated our option to employ cryolipolysis, which shows that cryolipolysis-induced temperatures have little or no everlasting effect on dermis and epidermis overlaying. Moreover, this cool ischemic damage may lead to adipose tissues cellular injury, lower activity of Na-K-ATPase, decreases adenosine triphosphate, increased levels of lactic acid and free radical mitochondrial release. The initial crystallisation and cold cryolipolysis insult are further worsened by ischemic-reperfusion injuries that generate reactive oxygen species, increase in cytosolic calcium grades and activate the apoptotic routes. **(20)**

Usually, these cells induce apoptosis and a severe inflammatory response by crystallising and cold ischemia damage, which will eventually remove the targeting adipocytes from the treated location in the next several weeks. **(16)**

The findings of **Zelickson etal., 2009** coincided with those of pigs as participants for the purpose of fat deletion by cryolipolysisconfirmation with histological analysis of mononuclear fatty cell loss adipocytes emergence, inflammatory transmission, and local fibrous septa thickness. **(21)Coleman et al.,2009** noticed that following a

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single cryolipolysis session, there was a decreasing layer of fat via ultrasound pictures.**(22) Stevens and Bachelor.2015** reported thatresults of ultrasonography data showed a marked reduction in fat layer for 40 patients who had undergone cryolipolysis. **(23)**

Our study results were compatible with **El-Desouky et al., 2016**who used cryolipolysis for two months and found a significant decrease of 5.8% in body weight, BMI showed a significant 5.83% reduction, WC was significantly reduced, and the SISF was significantly reduced by 17.41% post-treatment. **(24)** This was not different from**Mohamed Serag and Mohamed Elshafey 2016**whoresults also found a significant reduction in WHR, SISF, and WC after eight weeks of using cryolipolysis in addition to a significant decrease in abdominal subcutaneous adipose tissue measured by MRI with a 0.001 p-value, This decrease was linked to crystallisation of targeting Adipocytes and a cold ischemic lesion causing death in those cells and a marked inflammatory reaction which resulted in the treatment site being eventually removed within weeks. **(16)**

Our results also came with those from the study done by **Stevens et al. 2013**which found out that simultaneously combining two separate actions; cryolipolysis through hypothermal action at a temperature below 0ºC and electro-stimulation using specific currents, has a slimming draining effect which enhances reduction of localized fat deposits and better distribution of their volume. **(19)**

Also, our results are entirely supported by**Krueger et al.,2014** who showed that their abdomen fat thickness was reduced by 1 cm or 40% following only one session of cryolipolysis without damages to the skin overlyand **Manstein et al., 2008** who reported a reduction in abdominal fat thickness measured by abdominal ultrasonography and explained this reduction to"lipid-laden mononuclear inflammatory cells and local thickening of fibrous septae at two weeks post-procedure implicating apoptosis and phagocytosis." **(25)**

Preciado.2008highlighted the decrease in the circumference in treated regions by reducing the fat thickness and explained it as fat-layer degradation caused by cryolipolysis resulting in a deadly apoptotic lesion of adipocytes from cold-temperature exposure.**(26)**

After termination of diet therapy, leptin amount was elevated but was much less than the control group. **(27)** A further recent study by **Sumithran et al. 2011** looked into other leptin levels in 50 overweight and obese people with deficient energy diets. The link between a decrease of weight and leptin level was established.**(28)** In obese participants, **Rosenbaum et al.,2002** evaluated body structure and leptin levels. They have shown that leptin levels are lowered following weight loss. **(29)**We found that throughout the procedure, which was combining with the low amount of leptin in participants using body encirclement devices, BMI and weight were lowered, in accordance with these data. In addition, after **Mohammad Zadeh et al., 2015** intervention with radiofrequency and cavitation, the bodily fat mass fell dramatically, with a significant association between a declined abdominal circumference and a waist circumference following treatment, associated with lowered leptin levels.**(30)**

However,**Brennan and Mantzoros.2007**indicated that leptin values were shown to link with body fat levels.**(31)**Furthermore, in the control group, we have found a substantial drop in the overall level of cholesterol, TG, and LDL undergoing dietary treatment alone, whereas in the case group, these changes really aren't significant statistically. In accordance with our results, multiple additional research shows that the lipid profile levels are not affected by this body contouring procedure. In addition, we found a considerable good impact for abdominal and waist circumferences in combining bipolar radiofrequency and ultrasound cavitation technologies. The main weakness of the study was its limited sample size and the lack of a single cryolipolysis group and a non-intervention control group. Further research of the Leptin amount as a sensitivity biomarker is needed in a broader multi-center context. **(31)**

One flank therapy led to fat reduction of 40g during 3-months described by **Garibyan et al.,2014** Thus, it is roughly 160g of fat that will be removed during this time when the abdomen and bilateral flanks are treated. **(17)**This might mean around 1.8 grams per day upon release. The usual American diet includes a minimal 75 g fat per day to put this figure into context. **(32)**In addition, the consumption of up to 261 g of fat a day is found to have no harmful impact or lab abnormality.**(33)**Individuals can securely metabolise 250g of fat per day with whole IV parenteral nourishment and with triglycerides have proved to be free of maladministration 300–450g of triglyceride every day without any adverse effects. **(34)**

Although the processed adipocytes discharged their stored fat significantly more soon, **JörgenNordenström et al**. remarked in 2006; there seems to be little probability of causing adverse effects. **(35)**

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